PB90-263211

Determination of Biodegradability Kinetics of RCRA Compounds Using Respirometry for Structure-Activity Relationships

(U.S.) Environmental Protection Agency, Cincinnati, OH

1990

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1. REPORT NO.	2.	3. PB90-263211
EPA/600/D-90/136		

4. TITLE AND SUBTITLE
DETERMINATION OF BIODEGRADABILITY KINETICS OF RCRA COMPOUNDS USING RESPIROMETRY FOR STRUCTURE-ACTIVITY RELATIONSHIPS

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

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8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
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10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Risk Reduction Engineering Laboratory - Cincinnati, OH
Office of Research & Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/14

15. SUPPLEMENTARY NOTES
Project Officer: Henry H. Tabak Com'l (513)569-7681 or FTS 684-7681. Publ. in the Proceedings of the 44th Industrial Wast Conference, May 9-11, 1989, Purdue University.

16. ABSTRACT
Electrolytic respirometry is attaining prominence in biodegradation studies and is becoming one of the more suitable experimental methods for measuring the biodegradability and the kinetics of biodegradation of toxic organic compounds by the sewage, sludge, and soil microbiota and for determining substrate inhibitory effects to microorganisms in wastewater treatment systems. The purpose of this study was to obtain information on biological treatability of the benzene, phenol, phthalate, ketone organics and of the Superfund CERCLA organics bearing wastes in wastewater treatment systems which will support the development of an EPA technical guidance document on the discharge of the above organics to POTWs. The paper discusses the experimental design and procedural steps for the respirometric biodegradation and toxicity testing approach for individual organics or specific industrial wastes at different concentration levels in a mineral salts medium. A developed multi-level protocol is presented for determination of the biodegradability, microbial acclimation to toxic substrates and first order kinetic parameters of biodegradation for estimation of the Monod kinetic parameter of toxic organic compounds, in order to correlate the extent and rate of biodegradation with a predictive model based on chemical properties and molecular structure of these compounds. Respirometric biodegradation/inhibition and biokinetic data are provided for representative RCRA alkyl benzene and ketone organics.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

PB90-263211

EPA/600/D-90/136

45 DETERMINATION OF BIODEGRADABILITY KINETICS OF RCRA COMPOUNDS USING RESPIROMETRY FOR STRUCTURE-ACTIVITY RELATIONSHIPS

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INTRODUCTION

Electrolytic respirometry is attaining prominence in biodegradation studies and is becoming one of the more suitable experimental methods for measuring the biodegradability and the kinetics of biodegradation of toxic organic compounds by the sewage, sludge and soil microbiota and for determining substrate inhibitory effects to microorganisms in wastewater treatment systems.

Biodegradation of toxic and hazardous organic compounds holds a great promise as an important fate mechanism in wastewater treatment and in soil detoxification. Information about the extent and rate of biodegradation is a prerequisite for informed decision making on the applicability of the biodegradation approach. Unfortunately, relatively little quantitative data are available from which engineering judgement can be made, because of the large effort required to assess biodegradation kinetics.

The ongoing biodegradation studies are concerned with the prediction of the biological fate of toxic organic compounds using electrolytic respirometry as an approach to measure the biodegradation of selected organic compounds and generate biokinetic data so that these can be ultimately used to establish a possible correlation between molecular substrate configuration (physical/chemical characteristics) and biomass activity (kinetic parameters) as an index of biodegradation. The experimental respirometry testing is also providing data on the concentration levels of toxic organics inhibitory to microbial activity.

Initially, the inter-laboratory, ring test, Organization of Economic Cooperation and Development (OECD) studies at the EPA laboratory, Cincinnati, Ohio, were undertaken to develop confirmatory respirometric biodegradability testing procedure. Respirometric biodegradability and biokinetic data were provided for the selected non-inhibitory and nonadsorbing compounds, tetrahydrofuran, hexamine, pentaerythritol, 1-naphthol, sodium benzene sulphinate, thioglycolic acid and the biodegradable reference compound, aniline.

Subsequently, similar electrolytic respirometry studies were initiated to determine biodegradation kinetic parameters for selected representative toxic compounds of varied classes of organics included in the Priority Pollutant, RCRA and Superfund CERCLA lists, and to demonstrate presence of any inhibitory effects of these organics of specified concentration levels on the sludge biomass and on the metabolism of biogenic compounds.

The objectives of the present study were to utilize the electrolytic respirometry oxygen uptake data to: 1) determine the biodegradability of selected RCRA alkyl, chloro and nitrobenzenes, phenols, phthalates, and ketones and representative CERCLA leachate toxic organics; 2) generate information on their acclimated times (t_o) and the initiation and termination time values for the declining growth

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phase (t_1 and t_2); 3) determine their first order kinetic parameters of biodegradation (specific growth rate constants for the exponential growth phase (μ) and for the declining growth phase (μ'); 4) estimate the Monod kinetic parameters (μ_m, K_s and Y_g) of these compounds without initial growth or growth yield assumptions; 5) demonstrate presence of any inhibitory effects of these compounds on the metabolism of biodegradable reference compound, aniline; and 6) to correlate the extent and rate of biodegradation of these compounds with a predictive model based on chemical properties and structure of these compounds.

The purpose of this study was to obtain information on biological treatability of the benzene, phenol, phthalate and ketone organics and of the Superfund CERCLA organics bearing wastes in wastewater treatment systems which will support development of an EPA technical guidance document on the discharge of the above organics to POTWs. The study was to generate basic information on the fate of CERCLA leachate organics during on-site treatment and biodegradation and inhibition data for pollutants found in Superfund site wastewater that could be discharged to POTWs. Respirometric biodegradability, biokinetic and inhibition data were generated for the selected RCRA benzene, phenolic, phthalate and ketone compounds and for the six CERCLA leachate organics (benzoic acid, di-n-butyl phthalate, phenanthrene, n-nitrosodiphenylamine, p-chloro-m-cresol and hexachlorobutadiene).

BACKGROUND

Measurement of oxygen consumption is one of the oldest means of assessing biodegradability. Time consuming manual measurement of oxygen uptake (dilution BOD measurements) was replaced gradually by a more direct and continuous respirometric method for measurement of oxygen consumption in biochemical reactions, for use in routine examination of sewage and in control of sewage treatment process.

A rather comprehensive review of the use of respirometers for the study of sewage and industrial waste and their application to water pollution problems was published by Jenkins in 1960.[1] Montgomery's review[2] of respirometric methods summarized the design and application of respirometers for determination of BOD.

The application of respirometry was gradually directed to research studies to assess the toxicity and biodegradation of specific wastes or compounds, to evaluate factors affecting biological growth and to provide an insight into nitrification reaction. Of the commercial respirometers which have been developed for respirometric studies, the electrolytic respirometers were shown to be most applicable for measurement and quantitation of biodegradation activity because they automatically produce oxygen as needed, thereby eliminating some of the limitations of other techniques and allowing output data to be collected automatically for direct recording and processing.[3-9] A recent detailed review of respirometric techniques and their application to assess biodegradability and toxicity of organic pollutants was published by King and Dutka.[10]

Most uses of electrolytic respirometry in biodegradability testing have been for screening purposes to measure the extent of biodegradation as a percentage of the theoretical oxygen demand exerted in some time period.[9,11-17] A more recent study by Painter and King[18] concluded that a procedure based on electrolytic respirometry was reliable for assessing biodegradability, and could serve as an adequate Level I screening test for biodegradability.[19]

A considerable amount of studies using electrolytic respirometry to determine the biodegradability of wastes and specific organics is available in published literature and significant data on biodegradation of pollutants based on oxygen uptake have been generated.[2,4,5,20-34]

There are many techniques that have been used to evaluate biodegradation kinetics and these were reviewed in detail by Howard *et al.*[35,36] and Grady.[37] These techniques utilize continuous, fed-batch and batch type reactors for providing data from which kinetic parameters can be evaluated. The use of batch systems in biotechnology and biological wastewater treatment represents a less labor intensive, less expensive and much faster way to model biokinetics.

The kinetic parameters obtained by the above techniques should be intrinsic, that is, dependent only on the nature of the compound and the degrading microbial community and not on reactor system used for data collection. If this condition is satisfied then the parameters obtained can be used for any reactor configuration and can be used in mathematical models to estimate the fate of toxic organics.

Batch techniques are successful in obtaining intrinsic kinetic parameters by applying non-linear curve fitting techniques to single batch substrate removal curves, provided initial conditions are selected with proper care.[38-42] Batch systems can be used with either acclimated or unacclimated biomass for providing kinetic data and require that samples be taken at discrete time intervals during the course of biodegradation.[24,43,44]

Measurement of oxygen consumption through electrolytic respirometry is a batch type technique which has been shown to be very promising for automating data collection associated with biodegradation and intrinsic kinetic parameters. Studies performed by Dojlido,[11] Larson and Perry,[24] Tabak *et al.*,[45] Oshima *et al.*[46] Gaudy *et al.*[47,48] and Grady *et al.*[49-51] have utilized the electrolytic respirometry approach to generate biodegradation kinetic data.

Dojlido[11] divided the oxygen uptake curve into seven different phases and then proposed an empirical model for each phase and evaluated the biodegradability and toxicity of a test compound by measuring empirical rate constants and time interval associated with each phase. Various phases were distinguished by identifying inflection points in the curve through the plots of the logarithm of the slope versus time.

Tabak *et al.*[45] and Oshima *et al.*[46] sought to capitalize on Dojlido's method of identifying inflection points in order to quantify more fundamental rate coefficients. In order to identify more fundamental (and therefore intrinsic) kinetic coefficients, they used the generalized concept of oxygen uptake by Gaudy and Gaudy.[52] Substrate removal was divided into exponential and declining phases separated by an inflection point, and the endogenous phase. They coupled substrate removal and cell growth to oxygen consumption by imposition of an electron balance and consequently were able to evaluate μ_m from oxygen consumption data up to the inflection point. They proposed the use of the lag time as an indicator of how difficult it is to achieve acclimation to a test compound and their respirometric studies were carried out with an unacclimated biomass.

The justification for using respirometry to obtain intrinsic kinetic lies in the concept of oxygen consumption as an energy balance.[52,53] This concept states that all of the electrons available in a substrate undergoing biodegradation must either be transferred to the terminal acceptor or be incorporated into new biomass or soluble microbial products. If the concentrations of the substrate, the products and the biomass are all expressed in units of chemical demand (COD), then the oxygen uptake can be calculated in a batch reactor from an equation relating oxygen uptake to substrate, biomass and soluble products.[49] Furthermore, since biomass growth and product formation are proportional to substrate removal, this suggests that an oxygen uptake curve can provide the same information as either a substrate removal curve or a biomass growth curve. This latter concept has recently been used by Gaudy *et al.*[47,48] to calculate biodegradation kinetics. Specific growth rates obtained from growth studies as slopes of plots of L_n (biomass concentration) or Ln(X) versus time at different substrate concentrations, compared favorably with those obtained from exponential phase of respirometric oxygen uptake curves as slopes of plots of L_n (d oxygen uptake/d time) or Ln (dO_u/dt) versus time.

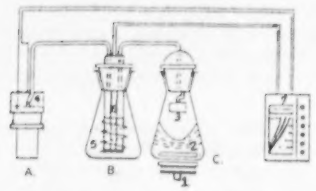
Studies of Grady *et al.*[49,50] have demonstrated that it is possible to determine intrinsic kinetics of single organic compounds by using only measurements of oxygen consumption in respirometric batch reactors. With the use of computer simulation techniques and non-linear curve fitting methods, intrinsic kinetic parameters were obtained from oxygen consumption data and were shown to be in agreement with those obtained from traditional measurement of substrate removal (DOC, SCOD, ^{14}C) or cell growth.

MATERIALS AND METHODS

Experimental Approach

The electrolytic respirometry studies were conducted using an automated continuous oxygen uptake and BOD measuring Voith Sapromat B-12 (12 unit system) electrolytic respirometer-analyzer. The instrument consists of a temperature controlled waterbath, containing measuring units, a recorder for digital indication and direct plotting of the decomposition velocity curves of organic compounds; and a cooling unit for the conditioning and continuous recirculation of waterbath volume. The recorder shows the digital indication of oxygen uptake and constructs a graph for these values of each measuring unit. The cooling unit constantly recirculates water to maintain constant temperature in waterbath. Each measuring unit as shown in Figure 1 is comprised of a reaction vessel with a carbon dioxide absorber mounted in a glass joint flask stopper, an oxygen generator and a pressure indicator. This measuring unit is interconnected by hoses, forming an air sealed system, so that the atmospheric pressure fluctuations do not adversely affect the results.

The activity of the microorganisms in the sample creates a vacuum which is recorded by the pressure indicator, which triggers the oxygen generator. The pressure conditions are balanced by electrolytic oxygen generation. The quantity of the sample, the amperage for the electrolysis and the speed of the synchronous motor are so adjusted that, with a sample of 250 mL, the digital counter indicates the oxygen uptake directly in mg/L. The CO_2 generated is absorbed by soda lime. The nitrogen/oxygen ratio in the gas phase above the sample is maintained throughout the experiment and there is no



A. Pressure indicator
B. Oxygen generator
C. Reaction vessel
1. Magnetic stirrer
2. Sample (250ml)
3. CO_2 absorber
4. Pressure indicator
5. Electrolyte
6. Electrodes
7. Recorder

Figure 1. Schematic diagram of measuring unit.

depletion of oxygen. The recorder-plotter concomitantly constructs an oxygen uptake graph for the selected values. The oxygen generators of the individual measuring units are electrolytic cells which supply the required amount of oxygen by electrolytic dissociation of a copper sulfate solution combined with sulfuric acid.

The nutrient solution used in these studies was an OECD synthetic medium *(OECD Guidelines for testing of chemicals*, OECD, Paris, France, 1981) consisting of measured amounts per liter of deionized distilled water of 1) mineral salts solution; 2) trace salts solution; and 3) a solution (150 mg/L) of yeast extract as a substitute for vitamin solution.

The microbial inoculum was an activated sludge from The Little Miami wastewater treatment plant in Cincinnati, Ohio, receiving municipal wastewater. The activated sludge sample was aerated for 24 hours before use to bring it to an endogenous phase. The sludge biomass was added to the medium at a concentration of 30 mg/L total solids. Total volumes of the synthetic medium in the 500 mL capacity reactor vessels were brought up to a final volume of 250 mL.

The test and control compound concentrations in the media were 100 mg/L. Aniline was used as the biodegradable reference compound, at a concentration of 100 mg/L.

The typical experimental system consisted of duplicate flasks for the reference substance aniline and the test compounds, a single flask for the physical/chemical test (compound control), a single flask for toxicity control (test compound plus aniline at 100 mg/L each) and an inoculum control. The contents of the reaction vessels were preliminarily stirred for an hour to ensure endogenous respiration state at the initiation of oxygen uptake measurements. Then the test compounds and aniline were added to it. The reaction vessels were then incubated at 25°C in the dark, enclosed in the temperature controlled waterbath and stirred continuously throughout the run. The microbiota of the activated sludge used as an inoculum were not pre-acclimated to the substrates. The incubation period of the experimental run was between 28 to 50 days. A more comprehensive description of the procedural steps of the respirometric tests is presented elsewhere.[19,45]

For fully automatic data acquisition, frequent recording and storage of large numbers of oxygen uptake data, the Sapromat B-12 recorders are interfaced to an IBM-AT computer via the Metrabyte interface system. The use of Laboratory Handbook software package allows the collection of data at 15 minute intervals.

Chemical Analysis

Indirect analysis of culture samples from respirometric vessels during the study included determination of chemical oxygen demand as soluble and total COD and of dissolved oxidizable carbon (DOC) with the use of Beckman Model 915 B system.

Specific substrate analysis of culture samples for the residual parent compound and the possible intermediate and final products of metabolism was performed with the use of Gas Chromatograph Varian 3700, equipped with FID and EC detectors and with different detector columns (depending on the substrate to be analyzed) and the Finnigan automated GC/EI-CI Mass Spectrophotometer system. Samples were analyzed as dichloromethane extracts of cultures (continuous liquid-liquid extraction) or by direct aqueous injection.

Suspended solids and biomass were measured gravimetrically (solids retained on a 0.45 μm filter) and by optical density determined spectrophotometrically (percent transmission read at 540 nm).

Determination of Substrate Biodegradability from Oxygen Uptake Data

In this study, biodegradation was measured by three approaches: *the first*, as the ratio of the measured BOD values in mg/L (oxygen uptake values of test compound minus inoculum control–endogenous oxygen uptake values) to the theoretical oxygen demand (ThOD) of substrate as a percent; *the second*, as a percentage of the test compound as measured by dissolved organic carbon (DOC) changes [OECD Guidelines for Testing of Chemicals (Method DGXI 283/82, Revision 5)[54]]; and *the third*, as a percentage of the test compound as measured by specific substrate analysis.

Graphical representations of percent biodegradation based on the BOD/ThOD ratio were developed against time for each test compound. The experimental DOC data for the initial samples and samples for reaction flasks collected at the end of experimental run were used to calculate the percent biodegradation based on the percent of DOC removal in the culture system.

Determination of Kinetic Parameters of Biodegradation

The Monod equation, relating cell growth to biomass and substrate concentration, and the linear law, relating cell growth to substrate removal, are the most popular kinetic expressions which can provide adequate description of growth behavior during biodegradation of substrate. Monod relation states that cell growth is first order with respect to biomass concentration (X) and mixed order with respect to substrate concentration (S) by the equation

$$dX/dt = (Su_mX)/(K_s + S) \quad (I)$$

Cell growth is related to substrate removal by the linear law by the equation

$$dX/dt = -Y(dS/dt) \quad (II)$$

The kinetics of biodegradation were evaluated by quantifying μ_m, K_s and Y kinetic parameters expressed in the *equation for the rate of substrate removal*, r_s:

$$r_s = -\mu_m X/Y \quad (1)$$

where X is the concentration of biomass capable of utilizing the organic substrate and Y is the biomass yield coefficient for the compound and in the *Monod equation*:

$$\mu = \mu_m S_s/K_s + S_s \quad (2)$$

(if the compound is not inhibitory to its own biodegradation) or by the *Haldane equation*:

$$\mu = \mu_m S_s/K_s + S_s + (S_s^2/K_I) \quad (3)$$

if the compound is inhibitory. In these equations, μ_m is the maximum specific growth rate, K_s is the half saturation coefficient, K_I is the inhibition coefficient and S is the concentration of substrate.

Determination of Rates of Exponential and Declining Growth. The *first order kinetic rate constants* (specific growth rate parameters) were determined by the linearization of the BOD curves or transforming the typical BOD curve to the linear function of time t, by the relationship of log dO_u/dt to t, which gives straight lines expressing the exponential and declining endogenous phases of the BOD curve as shown in Figures 2 and 3. The slope of the Ln(d oxygen uptake/dt) versus t give specific rate constants of the exponential growth phase (μ values) and the declining growth phase (μ' values) of the BOD curve as described by Dojlido,[11] Tabak *et al.*,[45] Oshima *et al.*,[46] and Tabak *et al.* [55,56]

Acclimation time values (t_o) and the time values for the initiation and termination of the declining growth phase (t_1 and t_2) for each test compound were determined from linearized expressions of BOD curves.

Estimation of Monod Kinetic Parameters. The *estimations of the Monod Kinetic parameters*, maximum specific growth rate constant, μ_m half saturation constant, K_s and growth yield constant, Y were determined directly from experimental oxygen uptake curves without the consideration of initial growth and growth yield assumption [Jobbagy, Grady and Tabak[57] and Tabak *et al.*[56]].

If the concentrations of the substrate, the products and the biomass are all expressed in BOD units, then the oxygen uptake (O_u at any time in the batch reactor may be calculated from

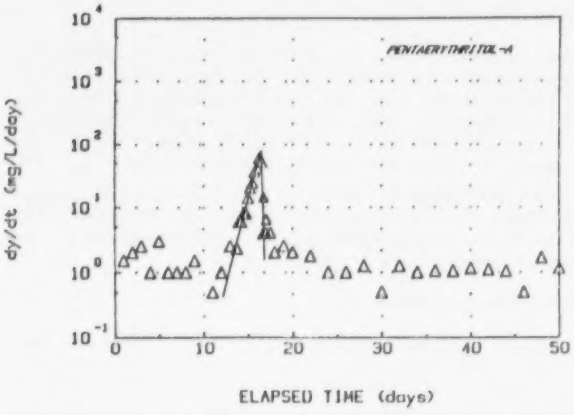


Figure 2. Log (dy/dt) versus t plot for pentaerythritol A.

$$O_u = (S_{so} - S_s) - (X - X_o) - (S_p - S_{po}) \quad (4)$$

where S_{so}, S_{po} and X_o are the concentrations of substrate, products and cells, respectively, at time zero, and S_o and S_p are the concentrations of substrate and product, respectively, at time t.

To apply Equation 4 for the determination of kinetic coefficients, equations must be available which express the concentrations of soluble substrate (S_s), soluble product (S_p) and biomass (X) as functions of time. For batch reactors, those equations are:

$$dS_s/dt = -(\mu_m/Y)S_sX/(K_s + S_s) \quad (5)$$

$$dS_p/dt = (Y_p\mu_m/Y)S_sX/(K_s + S_s) \quad (6)$$

$$dX/dt = \mu_m S_sX/(K_s + S_s) - K_sbX/(K_s + S_s) \quad (7)$$

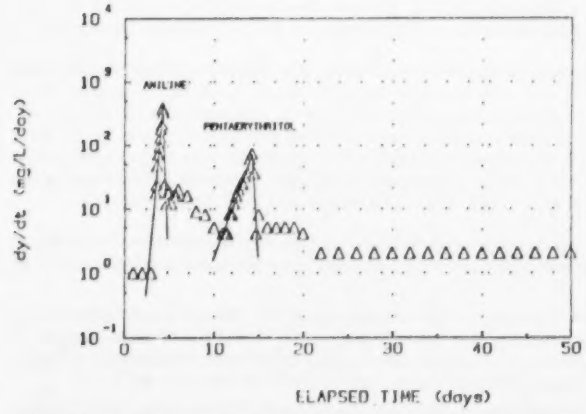


Figure 3. Log (dy/dt) versus t plot for pentaerythritol and aniline.

where S_s = soluble substrate concentration; S_p = soluble product concentration; Y_p = product yield, and b = decay coefficient.

To calculate oxygen uptake in a batch reactor, Equations 5, 6 and 7 must be solved simultaneously, and the resulting values of S_s, S_p and X over time substituted into Equation 4.

Determination of Y Constant. Y—the true yield parameter or the ratio of growth of biomass to substrate utilization, can be obtained from the experimental oxygen uptake curve at the initiation of the plateau of the curve as shown in Figure 4, with the use of equation:

$$Y = (1 - O_{upt}/S_o) - Y_p \tag{8}$$

A vertical line is drawn at the point of intersection of the tangents of the exponential and plateau phases of the curve. The oxygen uptake value obtained at the point of intersection of the vertical line (drawn through intersection of tangents) and oxygen uptake curve is the O_{upt} value [cumulative oxygen uptake value at the initiation of plateau].

The O_{upt} value is then plugged into the Equation 8, $Y = (S_o - O_{upt}/S_o) - Y_p$, where S_o is initial concentration of substrate and Y_p is soluble product concentration formed divided by initial substrate concentration, for Y determination. In this study, product yield (Y_p) was negligible.

Determination of μ_m Constant. The initial estimate of μ_m is obtained by the technique of Gaudy *et al.*[47,48] If Y_p is assumed to be zero, Equation 6 is eliminated and if b (decay constant) is assumed to be zero, Equation 7 is simplified to

$$dX/dt = (\mu_m S_s X)/(K_s + S_s) \tag{9}$$

Combining Equation 5 and 9 and integrating from S_{so} to S_s and from X_o to X gives:

$$X = X_o + Y(S_{so} - S_s) \tag{10}$$

If the assumption that $S >> K_s$, the term $S_s/K_s + S_s$ in Equations 5, 9 or I approaches 1, and these systems can be simplified through the use of Equation 10 to give equation

$$dX/dt = \mu_m X \tag{11}$$

Integrating Equation 11 and combining with equation (II) and then substituting in Equation 4 with P and P_o both equal to zero gives

$$Ln[X_o + O_u/(1/Y) - 1] = Ln(X_o) + \mu_m(est) \tag{12}$$

The plot of $Ln[(X_o + O_u)/(1/Y) - 1)]$ versus time will give a straight line with slope μ_m. The accuracy of μ_m(est) will depend upon the size of K_s, but this value is good enough as an estimate.

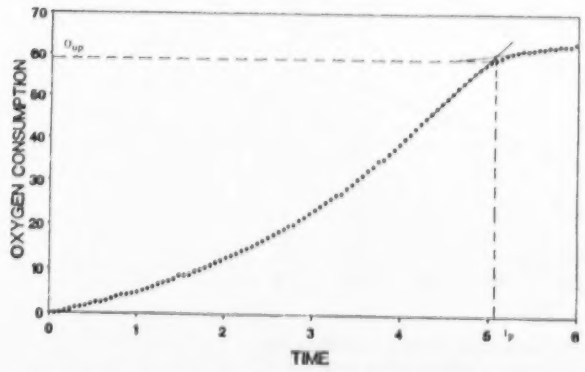


Figure 4. Determination of the start of the plateau in oxygen consumption for use in estimating y.

μm – the maximum specific growth rate – can be determined from experimental oxygen uptake curve plot in the following manner:

1. Values of the change of O_u with time (dO_u/dt) or slopes are determined along the entire experimental oxygen uptake curve as shown in Figure 5.
2. These dO_u/dt (slope) values are then plotted against the cumulative Ou values for each time interval, as shown in Figure 6.
3. The slope of the developed linearized form of oxygen uptake curve is the estimated μ_m value.

Determination of K_s Constant. K_s – the half saturation constant or the substrate concentration at which the specific growth rate is one-half the maximum specific growth rate – can be obtained from the experimental oxygen uptake curve in the following manner:

1. Value of O_{ul} can be calculated from the plot of (dO_u/dt) versus O_u provided the K_s value is 1 or less (insignificant in comparison to S_o value) and the plot contains a linear section with the slope μ_m, as shown in Figure 6.
2. Other (dO_u/dt) versus O_u plot in which the slope deviates from μ_m because of larger K_s values (more significant in comparison to S_o) is illustrated in Figure 7.

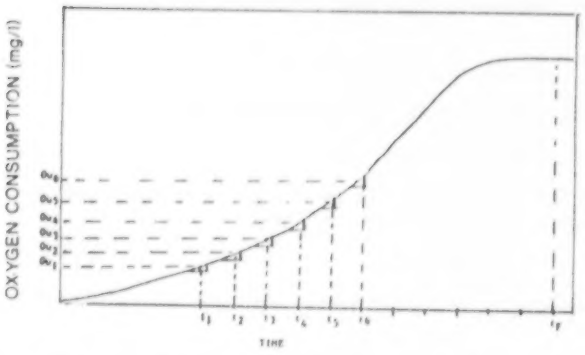


Figure 5. Plot of oxygen uptake rate (Ou versus time).

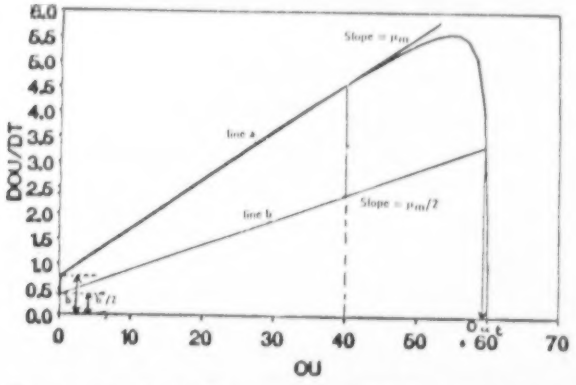


Figure 6. Plot showing (ΔOu/Δt) versus Ou_t curve containing a linear section with a slope μ_m to be used in estimating the values of K_s in cases where K_s values are negligible in comparison to S_o [μ_m = 0.1; K_s = 1; Y_g = 0.4; X_o = 5; S_o = 100].

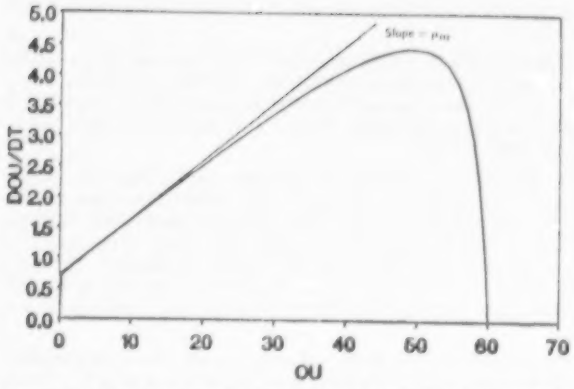


Figure 7. Plot of ($\Delta Ou/\Delta t$) versus Ou_t in which the slope deviates from μ_m because K_s values are more significant in comparison to S_o. [μ_m = 0.1; K_s = 5; Y_g = 0.4; X_o = 5; S_o = 100].

3. The value of dO_u/dt is determined at the intercept of the straight line developed from the plot of dO_u/dt versus O_u (Figure 6) which contains a linear section with slope μ_m.
4. Beginning with the value of 1/2 the intercept value, another straight line (b) is constructed with the slope 1/2 that of the slope of original line (a) whose slope is μ_m.
5. At the point where line (b) intercepts the declining experimental curve of the plot, a vertical line from that point of interception can provide the value of O_{ut} on the $\times$ axis.
6. This O_{ut} value is then used in the determination of K_s with the use of the equation

$$S_t = S_o - O_{ut}/(1 - Y_p - Y) = K_s \qquad (13)$$

where S_o = initial substrate concentration and S_t = substrate concentration at time t.

7. When the O_{ut}, Y, Y_p, and S_o values are plugged into the equation, value of S_t can be calculated, which is the value of K_s (in systems where K_s value is 1 or less).

Thus the oxygen uptake value O_{ut} associated with one-half of the estimate of μ_m is used in $K_s = S_o - O_{ut}/(1 - Y_p - Y)$ to get the estimate of K_s. The major impact of K_s is upon the shape of the oxygen uptake curve in the region of the plateau. Comparison of the experimental curves to a family of standardized curves as initial estimate provides an initial estimate of K_s that is sufficient for non-linear curve fitting techniques for quantitation of the kinetic parameters.

Quantitation of Monod Kinetic Parameters. The methodology for quantitation of the Monod kinetic parameters requires the use of above specific methods for estimating them initially and subsequently followed by computer simulation methods coupled with non-linear curve fitting techniques and is based on the use of measured values of initial growth and growth yield. The method requires the use of kinetic equation relating growth rate of biomass in presence of substrate, the substrate utilization rate, product formation rate and rate of oxygen consumption from O_2 uptake (BOD) curves to calculate and use the theoretical oxygen consumption data to quantitate the biokinetic parameters.

The determination of the kinetic parameters associated with biodegradation requires a series of steps. The initial substrate (S_{so}) and biomass (X_o) concentration must be carefully measured in COD units. The ratio of the two values must lie in a certain range in order to allow independent evaluation of μ_m, K_s and Y.[34] Grady's studies[18] have shown that a S_{so}/X_o ratio of around 20 works well. The value of Y_p may be estimated by determining the residual stable SCOD concentration after substrate depletion (plateau area). It is numerically equal to the residual SCOD divided by the initial SCOD. The value of the decay coefficient, b, may be determined by fitting to the oxygen consumption curve after the plateau when the only activity contributing to oxygen consumption is endogenous metabo-

lism and cell decay. Once X_o, S_{so}, Y_p and b are known, μ_m, K_s and Y may be determined by nonlinear curve fitting techniques.[18]

The technique involves the calculation of a theoretical oxygen consumption using oxygen uptake Equation 4 and Equations 5, 6, and 7 for substrate, product and biomass concentrations with assumed Monod parameters. The residual sum of squared errors (RSSE) associated with the difference in calculated and experimental oxygen uptake values is used to obtain new estimates. Above procedure is repeated till minimum RSSE is found.

The Grid Search technique was selected as a most suitable non-linear curve fitting technique for application in the determination of the kinetic parameters from oxygen uptake data, because it can allow easy discrimination between local minima and the global minimum RSSE. This technique enables a comparison between the calculated and experimental oxygen uptake data. The value of Y is fixed. For this value of Y, a pair of μ_m and K_s which give RSSE is found on a μ_m:K_s plane. The above procedure is repeated with other values of Y. Values of μ_m, K_s and Y which give minimum RSSE associated with the difference in calculated and experimental oxygen uptake data constitute the best values of the kinetic parameters.

The values of μ_m, K_s and Y developed from grid search technique, which when substituted into Equations 5–7, will provide X and S values, which (when substituted into oxygen uptake Equation 4) will in turn provide calculated oxygen uptake values at the region of the plateau, closest to the experimental oxygen uptake values, with a minimum RSSE, will constitute the best quantitative kinetic parameter values.

RESULTS AND DISCUSSION

Respirometric biodegradability, biokinetic and Monod kinetic data for selected RCRA alkyl, chloro- and nitrobenzenes and aliphatic and cyclic ketones are reported in this chapter. The electrolytic respirometry oxygen uptake data for the test compounds, the control reference compound, aniline, the inhibition and endogenous control systems were generated, revealing the lag phase (acclimation phase), the biodegradation (exponential) phase, the different bio-reaction rate slopes (characteristic of the test compound) as well as the plateau region at which the bio-oxidation rate reaches that of the endogenous rate of microbial activity. Figure 8 illustrates a representative oxygen uptake curve for aniline and the endogenous controls.

According to the OECD interpretation of biodegradation (BOD) data, test compounds were judged to be easily biodegradable if they showed a high level of oxygen uptake within ten days after the observed level of biodegradation first exceeds 10% (the time at which 10% biodegradation is achieved is considered the lag or acclimation period). In addition the results of biodegradation are valid if the control reference substrate, aniline achieved 60% biodegradation within a period of 28 days.

Based on the biokinetic equations relating growth rate of microbiota in presence of above compounds, the substrate utilization rate, and rate of oxygen uptake (BOD) curves, specific growth rate

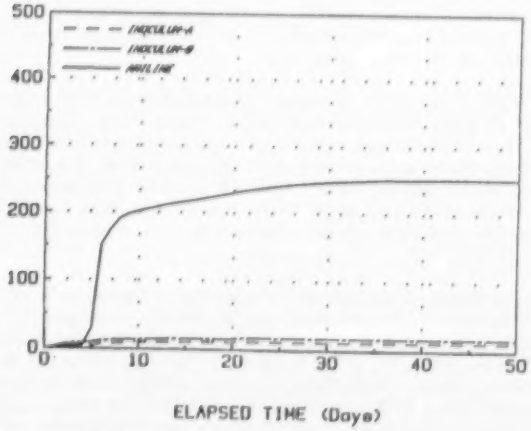


Figure 8. Oxygen uptake data on aniline.

kinetic parameters (biodegradation rate constants) were derived as slope values of the linearized plots (plots of the log of dO_u/dt) of exponential and declining growth phases of the BOD curve. The acclimation time values (t_o), and time values for the initiation and the termination of the declining growth phase (t_1 and t_2) for the test compounds and aniline were also generated.

The estimations of the Monod kinetic parameters for benzene and ketone compounds reported here, were determined directly from experimental oxygen uptake curves without the consideration of initial growth and growth yield assumption.

Respirometric Studies with Selected RCRA Alkyl, Chloro-, and Nitro-Benzene Compounds

The biodegradation of benzene, toluene, ethyl benzene, m- and p-xylenes, tert-butyl benzene, sec-butyl benzene, butyl benzene, cumene, 1-phenyl benzene and the reference compound, aniline at 100 mg/L concentration by 30 mg/L sludge biomass (as measured by oxygen consumption by sludge microbiota in mg O_2/L) was followed over a period of 20 days. The electrolytic respirometry oxygen uptake and BOD curves were generated and graphical treatment of the percent biodegradation was established for each compound. Figures 9 and 10 demonstrate typical oxygen uptake and BOD curves for toluene, toluene + aniline, p-xylene and p-xylene + aniline respectively,and Figures 11 and 12 illustrate graphically the percent biodegradation of toluene and p-xylene with time.

The percent biodegradation data based on the BOD/ThOD ratios for benzene, toluene, ethyl benzene, m- and p-xylene and the reference compound, aniline, are summarized in Table I. All of the above alkyl benzene compounds were shown to be biodegradable substrates at concentration levels of 100 mg/L when exposed to 30 mg/L of activated sludge biomass under the environmental conditions of the respirometric testing procedure, and within the period of 20 days of incubation.

The toxicity test control flask respirometric data revealed no inhibitory effects by these test compounds at the 100 mg/L concentration levels on the bio-oxidation of aniline by sludge microbiota.

Table II summarizes the bio-kinetic data for the benzenes studied, showing the specific growth rate constants for the exponential growth phase (μ values) and for the declining growth phase (μ' values) of the linearized form of the BOD curves of these compounds, as well as the t_o, t_1, and t_2 kinetic parameters. Figure 13 shows a typical plot of Ln (dO_u/dt) vs. time for toluene, from which the kinetic parameters were determined.

Table III summarizes the Monod kinetic parameter (μ_m, K_s, Y_g) data for these benzene compounds.

The biodegradation of monochlorobenzene, o- and m-dichlorobenzenes and the reference compound, aniline at 100 mg/L concentrations by 30 mg/L sludge biomass (as measured by oxygen consumption by sludge microbiota in mg O_2/L) was followed over a period of 50 days. Biodegradation data based on oxygen consumption (BOD) of the three chlorobenzenes revealed absence of bio-

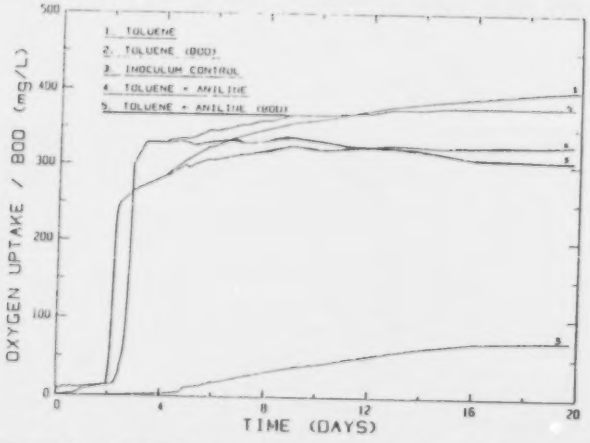


Figure 9. Biochemical oxygen uptake and BOD data for toluene and toluene + aniline.

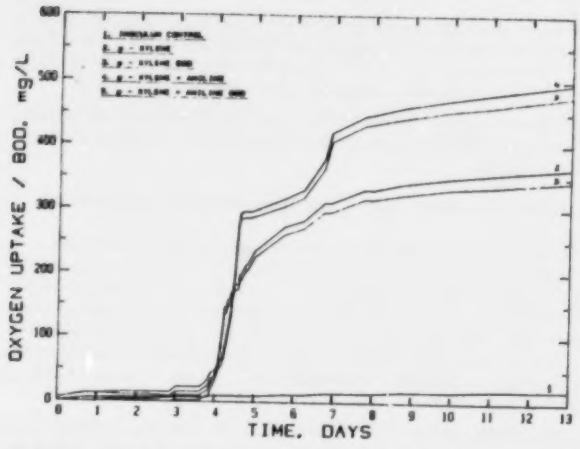


Figure 10. Biochemical oxygen uptake and BOD data for p-xylene and p-xylene + aniline.

activity of the sludge microbiota on these compounds in concentration levels of 100 mg/L and under the environmental conditions of the testing procedure and within the period of 50 days incubation. The sludge biomass was incapable of acclimating to the 100 mg/L concentration levels of the three chlorobenzenes in which the test compounds served as sole source of carbon in the reaction vessels. The oxygen uptake velocity curves for monochlorobenzene and m-dichlorobenzene followed that of the endogenous oxygen uptake rate of the inoculum control culture systems, with data points within the range of the endogenous level of respiration, whereas the oxygen uptake curves for o-dichlorobenzene was shown to run slightly below that of the endogenous oxygen uptake rate, possibly showing slight toxicity to the microbiota when the test compound is present in the reaction vessels as a sole carbon source at 100 mg/L concentration level.

The toxicity test control flask respirometric data revealed no toxic effects by the three chlorobenzenes at the 100 mg/L concentration levels on the bio-oxidation of aniline by sludge microbiota as shown by the complete biodegradation of aniline within the 50 days incubation period in presence of either of the above compounds. However, their presence in aniline culture flasks, causes a variable and pronounced effect on the acclimation to aniline biodegradation, as demonstrated by the extended

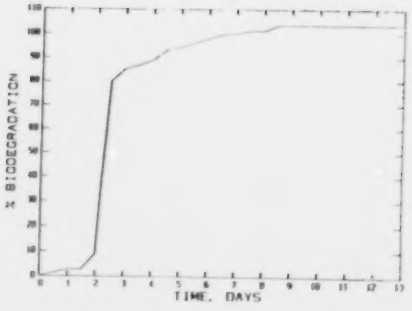


Figure 11. Biodegradation data for toluene.

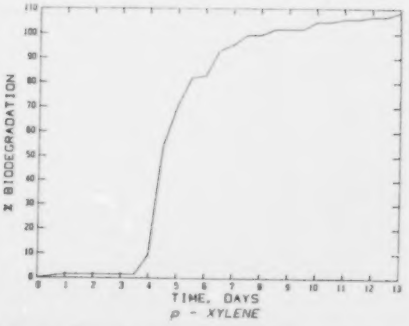


Figure 12. Biodegradation data for p-xylene.

Table I. Summary of Respirometric Biodegradation Data for Selected Benzenes

Time (days)	Percent Biodegradation (Based on % BOD Removal)					
	Aniline	Benzene	Toulene	Ethylbenzene	m-Xylene	p-Xylene
0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0	2.58	2.11	2.78	2.27	1.35	1.58
2.0	3.43	2.11	8.81	2.27	2.14	1.58
3.0	3.94	4.15	85.11	2.99	69.68	1.58
4.0	14.95	4.97	89.04	3.78	69.68	9.4
5.0	102.0	71.5	94.34	4.29	76.68	70.38
6.0	102.0	74.22	97.92	40.25	82.58	82.68
7.0	102.0	81.85	100.0	73.91	84.67	95.58
8.0	102.0	93.37	101.18	73.78	87.03	99.49
9.0	102.0	93.89	103.48	83.94	87.79	101.76
10.0	102.0	95.45	103.48	88.86	90.12	104.7
11.0	102.0	95.45	103.48	88.86	90.15	105.78
12.0	102.0	96.13	103.48	89.65	90.97	106.7
13.0	102.0	97.46	103.48	91.00	91.80	108.6

lag time before the start of exponential growth phase and degradation in the toxicity control systems containing aniline with either o-or m-dichlorobenzene.

Monochlorobenzene did not demonstrate any inhibitory effect on the acclimation time of biomass to aniline degradation in the chlorobenzene-aniline toxicity control flasks.

The biodegradation of nitrobenzene, 2,4- and 2,6-dinitrotoluenes, o and m-dinitrobenzenes, and p-nitroaniline at 100 mg/L concentrations in synthetic mineral salts media with 30 mg/L sludge biomass, as measured by oxygen uptake, was followed over a period of 50 days. Biodegradation data reveal absence of microbial activity on these compounds at 100 mg/L concentration levels and under the environmental conditions of the laboratory procedure. The toxicity tests data reveal that except for o-dinitrobenzene, none of the above compounds showed any toxic effects on aniline biodegradation at 100 mg/L concentration.

Respirometric Studies with Selected RCRA Ketone Compounds

Respirometric oxygen uptake data from the studies with the selected ketone compounds, acetone, 2-butanone, 4-methyl-3-pentanone and a cyclic ketone, isophorone were utilized to determine their biodegradability and biodegradation kinetic parameters.

All the ketones were shown to be biodegradable at 100 mg/L concentration levels in media containing 30 mg/L biomass and did not exhibit any toxicity to aniline biodegradation at these concentra-

Table II. Summary of Bio-kinetic Data for Selected Benzene Compounds

Compounds	ThOD for 100 mg	t_0 (days)	t_1 (days)	t_2 (days)	μ (day^{-1})	μ' (day^{-1})
Aniline						
(Experiment 1)	310	4.00	4.70	4.83	2.78	3.29
(Experiment 2)	310	4.00	4.65	4.79	3.80	8.60
Benzene	308	4.50	4.87	5.00	8.57	25.30
Ethyl benzene	317	4.00	4.21	4.83	8.33	44.80
Toulene	313	2.00	2.20	2.42	8.75	14.93
p-Xylene	317	3.90	4.22	4.83	9.94	4.50
m-Xylene	317	2.00	2.35	2.50	6.60	29.60
tert-Butyl benzene	322	4.40	5.12	5.70	1.21	1.69
sec-Butyl benzene	322	3.50	4.00	5.70	0.78	0.73
Cumene	320	2.40	2.79	3.00	2.31	2.24
Butyl benzene	322	3.30	3.92	4.56	2.42	2.42
1-Phenyl hexane	326	4.00	4.55	5.15	1.85	1.90

μ = specific growth rate constant for exponential growth phase of BOD curve.
μ' = specific growth rate constant for declining growth phase of BOD curve.

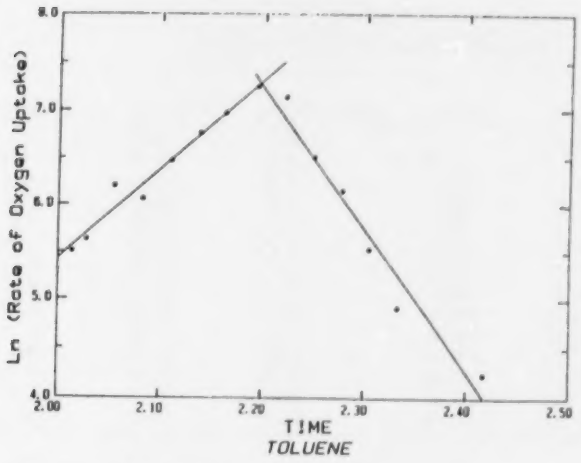


Figure 13. Plot of log ($\Delta Ou/\Delta t$) for toluene.

tions. Figures 14, 15, and 16 demonstrate typical oxygen uptake curves for acetone, 2-butanone and 4-methyl-2-pentanone and for each of these ketones in presence of aniline, respectively.

Tables IV and V summarize respectively the first order and Monod kinetic parameter data for these ketones.

CONCLUSIONS

The experimental data of respirometric studies with several classes of organic compounds definitely demonstrate that it is possible to measure the biodegradability (percent biodegradation as a ratio of BOD to ThOD) and to determine the kinetics of degradation of single organic compounds by using only measurements of oxygen consumption in respirometric batch reactors. The values of the kinetic parameters determined from oxygen consumption data were demonstrated to be similar to those based on the measurements of substrate removal and those made with cell growth data.

The generated data on biodegradation, biodegradation rates and substrate inhibition kinetics through the use of electrolytic respirometry, will enable the classification of biodegradability of toxic

Table III. Summary of Monod Kinetic Parameter Data for Selected Benzene Compounds

Compounds	Lag Time (t_0) days	Y_g mg biomass / mg substrate	μ_m (day^{-1})	K_s mg/L
Aniline	4.00	0.38	6.15	6.10
Benzene	4.50	0.23	9.30	22.16
Ethyl benzene	4.00	0.23	9.00	11.81
Toulene	2.00	0.20	9.09	56.74
p-Xylene	3.90	0.26	8.33	67.00
m-Xylene	2.00	0.14	11.50	35.61
tert-Butyl benzene	4.40	0.66	1.80	69.04
sec-Butyl benzene	3.50	0.64	2.39	22.00
Cumene	2.44	0.66	2.44	87.64
Butyl benzene	3.30	0.80	5.17	34.50
1-Phenyl hexane	4.00	0.67	2.15	35.09

μ = maximum specific growth rate.
K_s = half saturation constant; concentration of substrate at $\mu_m/2$.
Y_g = growth yield, mg biomass formed/mg substrate consumed.

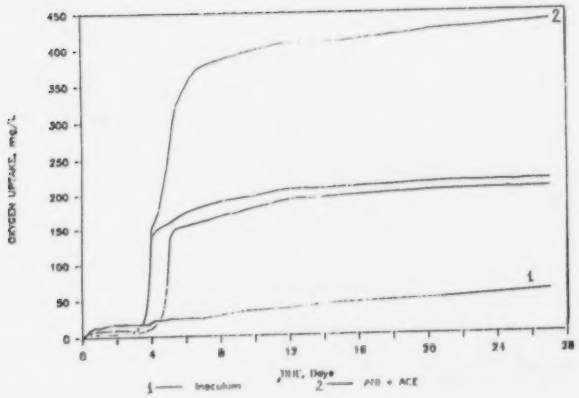


Figure 14. Oxygen uptake for acetone.

priority pollutant and RCRA toxic organic compounds and ultimate projection of the fate of organic compounds of similar molecular structure to those experimentally studied by way of the established predictive treatability models based on structure-activity relationships.

With the electrolytic respirometry approach, a data base on the removal of the above compounds by biodegradation fate mechanism can be adequately generated to support the development of predictive models on fate and removal of toxics in industrial and municipal waste treatment systems. A possible relationship between the kinetic parameters and the effect of different factors on these parameters, as determined through electrolytic respirometry and the structural properties of the organic pollutant, can eventually facilitate prediction of the extent and the rate of biodegradation of organic chemicals in the field of wastewater treatment systems from the knowledge of the structural properties of the pollutant organics.

A preliminary predictive biodegradation–structure/activity model based on the group contribution approach–was developed from the generated biodegradation kinetic data (first order kinetic parameters) with the use of electrolytic respirometry. It is expected that the model will closely predict the results found experimentally. In this way, the fate of other organic compounds may be anticipated without the time and expense of experimental work.

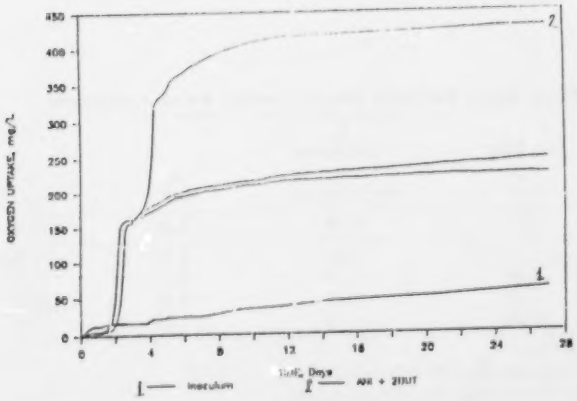


Figure 15. Oxygen uptake for 2-butanone.

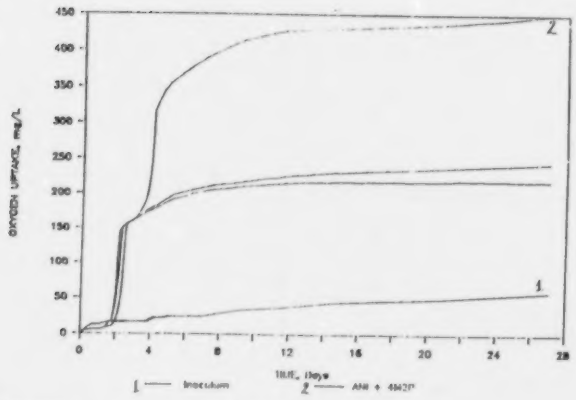


Figure 16. Oxygen uptake for 4-methyl-2-pentanone.

Studies are currently in progress to determine the effect of temperature, and different sources of sludge biomass (domestic and industrial wastewater treatment) on the biodegradation kinetics derived from electrolytic respirometry.

Table IV. Summary of Bio-Kinetic Data for Selected Ketone Compounds

Compounds	ThOD for 100 mg	t_0 (days)	t_1 (days)	t_2 (days)	μ (day^{-1})	μ' (day^{-1})
Aniline	310	4.00	4.70	4.83	2.78	3.29
Acetone	221	3.70	3.99	4.18	2.45	3.98
2-Butanone	244	2.00	2.20	2.35	2.41	4.98
4-Methyl-2-pentanone	272	1.85	2.24	2.35	2.31	4.80
Isophorone	278	22.30	23.70	25.40	0.73	0.38

μ = specific growth rate constant for exponential growth phase of BOD curve.
μ' = specific growth rate constant for declining growth phase of BOD curve.

Table V. Summary of Monod Kinetic Parameter Data for Selected Ketone Compounds

Compounds	Lag Time (t_0) days	Y_g mg biomass / mg substrate	μ_m (day^{-1})	K_s mg/L
Aniline	4.00	0.38	6.15	6.10
Acetone	3.70	0.36	4.86	9.76
2-Butanone	2.00	0.39	5.11	10.79
4-Methyl-2-pentanone	1.85	0.45	6.40	24.70
Isophorone	22.30	0.43	1.57	27.42

μ = maximum specific growth rate.
K_s = half saturation constant; concentration of substrate at $\mu_m/2$.
Y_g = growth yield, mg biomass formed/mg substrate consumed.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. Rena M. Howard and Mrs. Diana L. Redmond, secretaries in the U.S. Environmental Protection Agency's Risk Reduction Engineering Laboratory, Cincinnati, Ohio, for their excellent and timely word processing skills in preparing this manuscript for presentation at the 44th Annual Purdue Industrial Waste Conference, West Lafayette, Indiana.

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